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Evaluating Soluble CD25 as a Diagnostic Biomarker for Severe Aplastic Anemia: A Retrospective Analysis

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Abstract

BACKGROUND/AIMS: Severe aplastic anemia (SAA) is a critical marrow failure syndrome characterized by pancytopenia and bone marrow failure (BMF). Soluble CD25, which is involved in immune regulation, has been proposed as a potential biomarker of SAA. This study evaluated the diagnostic performance of soluble CD25 for differentiating SAA from other BMF failure syndromes.

MATERIALS AND METHODS: This retrospective study included 150 patients: 50 with SAA and 100 with other BMF syndromes. Demographic, clinical, and laboratory data were obtained, and soluble CD25 was measured using an ELISA kit. The diagnostic accuracy of soluble CD25 was assessed using receiver operating characteristic curve analysis.

RESULTS: The soluble CD25 concentration was significantly greater in the SAA group (3245 pg/mL) than in the BMF syndrome group (1250 pg/mL) ($p<0.001$). Soluble CD25 showed excellent diagnostic performance, with an area under the curve of 0.92 (95% confidence interval: 0.89-0.95). Using a cut-off value of 2500 pg/mL, the sensitivity was 84%, the specificity was 88%, the positive predictive value was 72%, and the negative predictive value was 93%. Soluble CD25 levels are correlated with disease severity, with higher levels linked to more severe cytopenia and lower bone marrow cellularity.

CONCLUSION: Soluble CD25 can be a useful non-invasive biomarker for the diagnosis of SAA because it is very accurate in distinguishing SAA from other marrow failure syndromes. The association of this parameter with disease severity makes it useful for early and accurate diagnosis, which enhances treatment.

Keywords: Soluble CD25, IL-2 receptor, severe aplastic anemia, bone marrow failure syndromes, diagnostic biomarker

INTRODUCTION

Aplastic anemia (AA) is a rare and frequently fatal disorder classified within the bone marrow failure (BMF) syndrome group. It is characterized by cytopenia and hypocellular bone marrow.[1] The incidence of AA displays two age peaks: childhood, at which age 10-25 years, is one such stage, and in elderly people, particularly those above 65 years. The global epidemiology of AA varies. In Western countries, the estimated annual incidence is 1. Five-2 cases per million people, whereas in Asia, the rate is 2-3 times greater, with 3.0-7.5 cases per million people. 0-7.[2] The cause of AA can be immunological, and its chief effect is on hematopoietic stem cells, which lead to BMF.[3,4] Therefore, the exact cause of the disorder has not yet been clearly defined, even though the disorder is believed to be polygenetic in origin, as indicated by the fact that several genes, environmental factors, and immune factors are known to be associated with its development.[5]

Severe aplastic anemia (SAA) is characterized by certain clinical and laboratory features that distinguish it from other types of AA. The criteria for diagnosis included a bone marrow cellularity of less than 25% and at least two of the following: a corrected reticulocyte count of less than 20×10^9/L, a platelet count of less than 20×10^9/L, and a neutrophil

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count of less than 0.5×10^9/L.[6,7] SAA may lead to massive bleeding and infection and increase the risk of clonal disorders, such as myelodysplastic syndrome (MDS) and acute myeloid leukemia.[8] Therefore, the diagnostic results appear to play a crucial role in the effective and timely delivery of care and the achievement of good treatment outcomes. The current diagnostic approach for AA involves the evaluation of the patient's medical and physical examinations and other investigations. Among the common diagnostic procedures for the classification of bone marrow disorders are peripheral blood count, bone marrow aspiration, and trephine biopsy.[9,10] For example, differentiating between AA and other types of BMF syndromes, such as MDS, paroxysmal nocturnal hemoglobinuria (PNH), and congenital marrow failure disorders, is very challenging because they all have similar clinical and pathological features.[11]

Interleukin-2 (IL-2) is a cytokine that plays a role in the immune response, and as demonstrated in other experiments, T-cell proliferation is an important aspect of immunity.[12,13] The IL-2 receptor (IL-2R) is composed of three subunits. The IL-2R complex contains three subunits: the IL-2R alpha subunit (CD25), the IL-2R beta subunit (CD122), and the IL-2R gamma subunit (CD132).[14] Soluble CD25 (sCD25) is a bioactive molecule that is a recombinant human soluble IL-2R alpha chain that is present in serum and participates in many immunological diseases, including AA.[15] Analysis of the results revealed a correlation between AA and sCD25, and the level of sCD25 in the plasma of AA patients is elevated. Because sCD25 blocks the effect of IL-2 on T-lymphocyte proliferation, it is expected to be implicated in the pathogenesis of this disease via immune dysfunction.[16] Additionally, sCD25 has been suggested as a biomarker of AA and for disease monitoring because sCD25 levels increase at disease onset and correlate with therapy.[16]

The soluble CD25 receptor is widely used in immunologic and inflammatory diseases.[17,18] The soluble CD25 receptor is elevated in infection, autoimmunity, hematological dyscrasia, and solid neoplasms.[16,19] Additionally, the soluble CD25 receptor has been proposed as a biomarker of lymphohistiocytosis and macrophage activation syndrome, which are conditions associated with immune regulatory dysfunction and inflammation.[20,21] Under such conditions, the level of soluble CD25 receptor is often elevated, and it is used as a diagnostic criterion for this disease.[20,21]

Soluble CD117, also known as soluble c-kit, is a potential biomarker for hematological malignancies.[22] Soluble CD117 is involved in hematopoiesis and, in particular, in the maintenance of stem cells.[23] Therefore, higher sCD117 concentrations in the serum of patients with hematological malignancies are associated with disease progression.[22,24] The relationship between sCD117 and immune-mediated BMF diseases, such as SAA has not been well explained. The level of sCD117 in SAA patients and its efficacy in the diagnosis of this disease are still insufficiently understood. Thus, sCD117 can be regarded as a marker of hematopoietic activity, and its levels are usually low in SAA patients.

Nevertheless, there is a lack of sufficient information regarding which part of sCD25 is best used to differentiate between AA and other types of BMF syndrome. Therefore, the present study aimed to improve the potential of sCD25 in differentiating SAA from other marrow failure syndromes. Thus, this study aimed to determine the level of sCD25 in SAA patients and controls and the sensitivity, specificity, and predictivity of this marker for the assessment of its diagnostic potential for non-invasive SAA.

MATERIALS AND METHODS

The study protocol was approved by the Dali Referral Institutional Review Board of Hospital Ethics Committee (approval number: 88/17, date: 20.11.2017). The study data were retrospectively collected from the Hematology Department of the Referral Hospital from January 2018 to December 2022. This single-center retrospective study aimed to investigate the diagnostic potential of soluble CD25 (sCD25) in patients with BMF syndromes, including SAA, MDS, PNH, and inherited BMF syndromes.

A total of 150 patients were included in the study, representing the total number of patients aged 18 years or older with BMF who attended the hospital during the study period. Among them, 50 patients were diagnosed with SAA, and 100 patients were diagnosed with other BMF syndromes (MDS, PNH, or inherited BMF syndromes). Patients were selected from hospital records based on specific inclusion criteria: all individuals diagnosed with BMF who had complete medical records available. The exclusion criteria were applied to exclude patients who had undergone hematopoietic stem cell transplantation, received immunosuppressive therapy within the last year, or had missing or incomplete medical records.

Descriptive analysis was used to present the demographic and clinical features of the study population. The diagnosis and classification of SAA, inherited BMF syndromes, MDS, and PNH were based on standard clinical and laboratory criteria. Information on coexisting viral infections, such as hepatitis B virus, hepatitis C virus, and severe acute respiratory syndrome-coronavirus-2, was also collected from both SAA and non-SAA patients.

Statistical Analysis

The levels of soluble CD25 (sCD25) were measured using a sensitive human-soluble CD25 ELISA kit. The ROC curve was generated to evaluate the diagnostic performance of sCD25 for SAA compared with other BMF syndromes. The area under the curve (AUC) was calculated, and Youden's index was used to identify the optimal cut-off point. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated for the cut-off point.

To explore the relationship between sCD25 levels and disease severity, the Kruskal-Wallis test was conducted, followed by Bonferroni's post-hoc correction. Spearman's rank correlation coefficient was used to assess correlations between sCD25 levels and other continuous variables, such as laboratory findings.

A two-tailed p-value 0.05 was considered statistically significant. Data analysis was carried out using SPSS Statistics 28 (IBM Corp., Armonk, NY) and R version 4.2.1 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Demographic and Clinical Characteristics

The present study included 150 patients, among whom 50 had SAA and 100 had other subtypes of BMF syndrome. The mean age of the SAA patients was 48 years. The mean age of patients with other syndromes was 43 years. 5±17. 2 years. The gender distribution was 1.2:1 for SAA and 1.6:1 for the other syndromes. The most common symptoms included (90% in SAA, 88% in other syndromes), easy bruising (76% in SAA, 65% in other syndromes), and recurrent infections (64% in SAA, 52% in other syndromes) as shown in Table 1.

Regarding the specific diagnoses within the other BMF syndrome groups, we observed the following distributions: MDS in 40 patients; inherited BMF syndrome in 35 patients; Fanconi anemia in 15 patients; Blackfan-Diamond anemia in 10 patients; D dyskeratosis congenita in 8 patients; and PNH in 25 patients. Laboratory findings revealed that the median soluble CD25 (sCD25) level in the SAA group was significantly greater at 3245 pg/mL [interquartile range (IQR): 2850-3620 pg/mL] compared to the other BMF syndrome group, which had a median sCD25 level of 1250 pg/mL (IQR: 980-1560 pg/mL) ($p<0.001$). The average laboratory values for neutrophils, erythrocytes, hemoglobin, platelets, C-reactive protein (CRP), and procalcitonin (PCT) in the SAA group and other groups were as follows: SAA group: neutrophils, 1.2±0.6x10^9/L; erythrocytes, 2.5±0.8x10^12/L; Hb, 8.6±1.5 g/dL; platelet (PLT), 18.4±7.2 x10^9/L; CRP, 12.4±5.3 mg/L; PCT, 0.08±0.04 ng/mL; and other group: neutrophils, 2.1±0.9x10^9/L; erythrocytes, 3.8±1.2x10^12/L; Hb, 11.2±2.5 g/dL; PLT, 120.6±45.3x10^9/L; CRP, 8.7±3.2 mg/L; and PCT, 0.05±0.02 ng/mL as shown in Table 2.

Diagnostic Performance of sCD25

sCD25 was effective in discriminating between SAA and other BMF disorders, with an receiver operating characteristic (ROC)-AUC of 0.92 (95% confidence interval: 0.89-0.95). Using a cut-off point of 2500 pg/mL, the sensitivity of the test was 84%, specificity was 88%, PPV was 72%, and NPV was 93%. Compared with patients with other syndromes, SAA patients with sCD25 levels above this threshold were significantly different ($p<0.001$).

Correlation with Disease Severity

In the context of SAA, 50 and 42 patients were treated with immunosuppressive drugs with the aim of reducing the immunological attack on hematopoietic stem cells. The treatment modality of hematopoietic stem cell transplantation was considered in patients with severe disease or relapse. In contrast, patients with other BMF syndromes received tailored therapies based on specific diagnoses. Therefore, in the group of patients with MDS (32 of 40), hypomethylating agents, including azacitidine or decitabine, were used, whereas supportive care with transfusions was mainly used for patients with inherited disorders, such as Fanconi anemia. In addition to treatment, some patients with FA receive androgens or hematopoietic growth factors to improve erythropoiesis. Out of the 25 patients with PNH, 20 were administered eculizumab, a medication that prevents the complement system from attacking red blood cells and causing thrombosis. Table 3.

Response to Immunosuppressive Therapy

This study also revealed a statistically significant relationship between sCD25 levels and the outcome of immunosuppressive treatment in SAA patients ($p=0.045$). The amount of sCD25 in the patient's blood plasma was greater in non-responders than in responders.

Comparison Between the New Diagnostic Marker and the Other Markers

Of the measured parameters in this study, sCD25 was considered superior to the reticulocyte and absolute neutrophil counts. The AUC for sCD25 was 0.92, which was significantly greater than the AUC of the reticulocyte count (0.76) and absolute neutrophil count (0.71). Thus, sCD25 had greater sensitivity and specificity than the other markers and could be useful for differentiating SAA from other types of BMF diseases. Table 4.

DISCUSSION

We aimed to establish the possibility of using sCD25 as a diagnostic marker for SAA and to compare it with that of other BMF syndromes. Finally, the data obtained in the present study strongly support the hypothesis that sCD25 can be used to enhance diagnostic strategies and therapeutic outcomes in SAA patients. Therefore, our study also

Table 1. Demographic and clinical characteristics

Characteristic	SAA group (n=50)	Other bone marrow failure syndromes group, (n=100)	Total, (n=150)
Mean age (years)	48.6 ± 12.3	43.5±17.2	45.2±15.6
Males, n (%)	28 (56%)	62 (62%)	90
Female, n (%)	22 (44%)	38 (38%)	60
Male-to-female ratio	1.2:1	1.6:1	1.2:1
Fatigue (%)	45 (90%)	88 (88%)	89
Easy bruising (%)	38 (76%)	65(65%)	69
Recurrent infections (%)	32(64%)	52 (52%)	57

SAA: Severe aplastic anemia.

Table 2. Laboratory findings

Parameter	SAA group	Other group
Neutrophils (x10^9/L)	1.2±0.6	2.1±0.9
Erythrocytes (x10^12/L)	2.5±0.8	3.8±1.2
Hemoglobin (g/dL)	8.6±1.5	11.2±2.5
Platelets (x10^9/L)	18.4±7.2	120.6±45.3
CRP (mg/L)	12.4±5.3	8.7±3.2
PCT (ng/mL)	0.08±0.04	0.05±0.02
sCD25 (pg/mL)	3245±420	1250±350

CRP: C-reactive protein, PCT: Procalcitonin.

Table 3. Correlations between sCD25 levels and disease severity

Parameter	Spearman's rho	p
Reticulocyte count	-0.56	<0.001
Absolute neutrophil count	-0.48	<0.001
Bone marrow cellularity	-0.39	0.003

Table 4. Comparison between the new diagnostic marker and the other markers

Marker	AUC	Sensitivity	Specificity
sCD25	0.92	84%	88%
Reticulocyte count	0.76	-	-
Absolute neutrophil count	0.71	-	-

AUC: Area under the curve.

reinforces the involvement of sCD25 in SAA, as patients with this disease present higher levels of this marker than those with other BMF disorders. This finding is consistent with other studies showing that sCD25 is a marker of immune dysregulation and the severity of various immunological diseases.[25,26]

The AUC of sCD25 was 0.92, indicating that it can distinguish SAA from other types of BMF syndrome. The selected cut-off criterion of 2500 pg/mL provides optimal sensitivity and specificity compared with those of other routine tests, such as reticulocyte and neutrophil counts. This is crucial from a clinical perspective because proper and early diagnosis of SAA is important for the initiation of appropriate management. The comparatively low sensitivity of sCD25 can be especially useful for excluding SAA and allowing clinicians to identify other possibilities. Consequently, this study links sCD25 to the severity of SAA in the patient group. A sCD25 level above the median is associated with a greater degree of cytopenia and lower bone marrow cellularity. In agreement with other findings, sCD25 could be used for the assessment of the disease state and the state of bone marrow.[16,27]

The association between sCD25 and the results of immunosuppressive treatment is important. These data suggest that SAA patients with elevated sCD25 levels may not require immunosuppressive treatment; thus, sCD25 can be used to predict treatment outcome and patient's need for HSCT. This finding has some therapeutic implications because it may help in the formulation of treatment plans for patients. Despite the findings of this study, further large-scale prospective studies should be conducted to determine whether sCD25 is useful for predicting patients who will benefit from immunosuppressive treatments.

Comparison with Other Markers

This study revealed that sCD25 had better diagnostic efficiency than common markers, such as reticulocyte and neutrophil counts. The higher AUC, sensitivity, and specificity of sCD25 are better than those of the others and can therefore help in the diagnosis of SAA, especially in cases where the clinical and pathological features are quite similar. The findings of this study suggest that sCD25 assessment can be useful in the diagnosis of BMF syndrome when the results are combined with other clinical and laboratory parameters.

Study Limitations

There are several limitations associated with this retrospective study that can be overcome in a prospective, large-scale multicenter study. Expanding the study of less frequent BMF subtypes may improve our knowledge of the value of sCD25 as a diagnostic marker. More studies are needed to determine the factors that increase sCD25 in patients with SAA and the effectiveness of this marker in disease progression.

CONCLUSION

Soluble CD25 (sCD25) is a non-invasive diagnostic biomarker of SAA. The good diagnostic sensitivity, association with disease severity, and possible ability to predict response to immunosuppressive treatment warrants its role in the management of SAA.

MAIN POINTS

- **Elevated sCD25 levels in SAA:** Soluble CD25 (sCD25) levels were significantly higher in patients with severe aplastic anemia (SAA) than in those with other bone marrow failure (BMF) syndromes, indicating its potential as a diagnostic marker.
- **Excellent diagnostic accuracy:** This study demonstrated that sCD25 had an excellent diagnostic accuracy for distinguishing SAA from other BMF syndromes, with an AUC of 0.92.
- **Correlation with disease severity:** Higher sCD25 levels were associated with more severe cytopenia and lower bone marrow cellularity, suggesting its role in assessing disease severity in SAA patients.
- **Predictive value for treatment outcomes:** Elevated sCD25 levels predicted non-responsiveness to immunosuppressive therapy in SAA patients, indicating its potential utility in guiding treatment decisions.
- **Superior to traditional markers:** sCD25 outperformed traditional markers like reticulocyte and neutrophil counts, in diagnosing SAA, offering a more sensitive and specific tool for clinicians.

ETHICS

Ethics Committee Approval: The study protocol was approved by the Dali Referral Institutional Review Board of Hospital Ethics Committee (approval number: 88/17, date: 20.11.2017).

Informed Consent: Retrospective study.

Footnotes

Financial Disclosure: The author declared that this study had received no financial support.

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